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RESPONSIVENESS OF LATERAL COMPARTMENT JOINT SPACE WIDTH: DATA FROM THE OSTEOARTHRITIS INITIATIVE (OAI)

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Purpose: An important structural measure of OA progression is radiographic joint space width (JSW). Although a substantial fraction of knees have lateral compartment OA, this compartment is much less studied. As an example, the knee radiographs for the OAI were acquired with a protocol designed for the medial compartment, therefore measurements of lateral compartment JSW are potentially less reliable. Furthermore, JSW measurement covering the full extent of the lateral compartment could not be made for a substantial fraction of the knees due to sub optimal positioning for the lateral compartment. Previous work has confirmed the most responsive location to measure JSW in the medial compartment as a function of x, a dimensionless variable derived from an anatomical coordinate system. An analogous location in the lateral compartment has not been determined, and currently lateral compartment JSW readings for the OAI have been deemed "experimental". The goal of this project was to determine the most responsive locations in the lateral compartment to measure JSW loss in patients with lateral compartment disease.
Methods: We selected all patients from the OAI with knee radiographs available for the baseline (BL) and 48 month visits, BL Kellgren Lawrence score of 2 or 3, and greater joint space narrowing in the lateral compartment than the medial compartment based on the OARSI JSN score. Exclusion criteria were the presence of TKR and inconsistent positioning of the knee as determined by the tibial alignment (OAI variable TPCFDS), a measure of the distance between the projected tibia rim and plateau. A change of greater than 2mm was grounds for exclusion. These criteria provided 326 knees from 278 unique IDs.
Results: Complete coverage of the tibial plateau (defined as $x = 0.700$ to 0.900) was available for 283 out of 326 knees (86%). A secondary analysis was performed including knees ($N = 300$, 92%) with partial coverage (defined as $x = 0.700$ to 0.775). (Row 5 of the table) The location $x = 0.725$ provided the most responsive measure of JSW for all groups, but the responsiveness was substantially uniform from $x = 0.700$ to $x = 0.775$.
Conclusions: In patients with lateral OA we found that the most responsive measure of JSW was in the inner portion of the compartment (between $x = 0.700$ and $x = 0.775$) as measured by the SRM value. In this range there was little variation in SRM value, so each location provides a comparably responsive measure. An examination of the two SRM components revealed that improved performance in the $x = 0.700$ to $x = 0.775$ was due to a reduced standard deviation rather than increased joint space loss. This suggests that this region provides a more reliable measurement rather than reflecting more change. This study marks a step toward understanding the best way to measure JSW in patients with lateral compartment knee OA.

Table. SRM values at fixed locations (x) along lateral compartment of the knee. N/A corresponds to missing values due to lack of coverage for all knees

	x = 0.700	**x = 0.725**	**x = 0.750**	**x = 0.775**	**x = 0.800**	**x = 0.825**	**x = 0.850**	**x = 0.875**	**x = 0.900**
All (*N* = 283)	-0.99	-1.06	-1.03	-0.98	-0.95	-0.90	-0.86	-0.83	-0.85
KL2 (*N* = 157)	-0.94	-0.99	-0.95	-0.87	-0.83	-0.80	-0.79	-0.79	-0.81
KL3 (*N* = 12 6)	-1.06	-1.16	-1.14	-1.15	-1.14	-1.07	-0.96	-0.90	-0.91
Including partial (*N* = 300)	-0.94	-1.00	-0.97	-0.92	N/A	N/A	N/A	N/A	N/A

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COMPREHENSIVE PROTEIN PROFILING OF SYNOVIAL FLUID IN OSTEOARTHRITIS

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Purpose: In order to understand the pathophysiology of osteoarthritis (OA) its mediators need to be identified. Synovial fluid (SF) is a joint located serum filtrate with additional contributions from articular cartilage, synovium and bone. It represents a potential source of disease specific proteins that could aid in the understanding of the pathogenesis of joint disease and be used in the early diagnosis of disease. Mass spectrometry (MS) provides opportunities to discover disease mechanisms whilst simultaneously identifying potential biomarkers of OA. In MS high abundance proteins interfere with the signal from low abundance proteins; the fraction containing most potential proteins of interest. To overcome this methods of protein depletion or equalisation have been used. However in SF proteomics only protein depletion using expensive immunodepletion columns has been implemented. We therefore investigated the use of a more cost effective equalisation method using Proteominer™ beads in order to comprehensively profile the protein complement of SF in health and OA using liquid chromatography mass spectrometry (LC-MS/MS). Additionally label-free quantification identified potential OA biomarkers.
Methods: Following the collection of SF from the metacarpophalangeal joints of 9 normal and 9 OA racing thoroughbred horses macroscopic, microscopic and synovitis scoring was undertaken. SF was hyaluronidase treated and high abundant proteins depleted using ProteoMiner™ equalisation beads. Reduction, alkylation and trypsin digestion were undertaken directly on the beads after equal protein loading. To investigate the efficiency of ProteoMiner™ technology a single SF sample was analysed without protein depletion. All samples were individually analysed on a two hour gradient with LC-MS/MS using a NanoAcquity LC coupled to a LTQ Orbitrap Velos. Progenesis™ LC-MS software was used for label-free quantification with data searched for protein identifications using Mascot in the Ensembl database for horse. To maximise the number of quantifiable protein with an acceptable false discovery rate (FDR) the peptide matches were adjusted to 1% FDR prior to the protein identifications being re-imported into Progenesis™. Adjusted ANOVA values of $p<0.05$ and additionally regulation of >2-fold were regarded as significant.
Results: The average modified Mankins score, palmar osteochondral disease score and synovitis scores for normal samples were 0.8 ± 0.35, 0 and 1.1 ± 0.2 and OA samples were 13.6 ± 1.4, 1.7 ± 0.2, 2.3 ± 0.2 (mean $\pm$ standard error mean). The number of protein identifications was increased by 33% in the Proteominer™ treated SF compared to undepleted SF. Following Proteominer™ treatment and Progenesis™ analysis a total of 754 proteins were identified in SF, 593 with a significant Mascot score. Thus Proteominer™ beads concentrated the lower abundance proteins enabling the most comprehensive SF proteome to date. Proteins identified included those relating to matrix proteins, inflammatory factors, complement activation proteins and proteases. A subset of 10 proteins were identified which were differentially expressed in OA SF (Table 1).
Conclusions: A number of proteins were identified for the first time in SF which may be involved in the pathogenesis of OA. We identified a distinct set of proteins that may act as potential biomarkers to distinguish between normal and OA joints. S100-A10, a calcium binding protein has upregulated in OA. Other S100 proteins have been demonstrated as having a role in the pathogenesis of OA and in SF proteomic studies. However this is the first time S100-A10 has been implicated in OA. Together with its binding partner annexin 2 it acts as a plasminogen receptor and regulates plasminogen-dependant macrophage activation. This may have a role in the synthesis and activation of matrix degrading proteases. CD109 is a TGF-β co-receptor, released from the chondrocyte cell surface that inhibits TGF-β signalling. Its contribution to the disregulation of TGF-β is unknown.

Proteins differentially expressed in OA SF identified with gretare than 2 fold change and $P<0.05$

Protein	Anova (p)	Maximum fold change	Highest mean condition
S100-A10	0.00028	2.2	OA
CD109 antigen	0.00039	2.0	OA
Phospholipid transfer protein isoform 1	0.00098	2.7	OA
Complement component C8 gamma chain	0.0115	3.1	OA
Collagen alpha-1(I) chain	0.0459	2.8	OA
Calsyntenin-1	0.0485	2.1	OA
Integral membrane protein 2B	0.00068	2.1	Normal
mannan-binding lectin serine protease 2	0.0061	2.2	Normal
Keratin, type II cytoskeletal 7	0.0114	2.2	Normal
Cyclin D binding myb-like transcription factor 1	0.0240	6.2	Normal

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SERUM BIOCHEMICAL MARKERS OF JOINT METABOLISM AND INFLAMMATION IN RELATION TO CLINICAL SYMPTOMS AND PHYSICAL FUNCTION IN ADULTS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS

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Purpose: To date, the majority of osteoarthritis (OA) biomarker research has examined associations between biomarker levels and radiographic parameters. While radiography provides information on the structural burden of disease, it is insensitive to small changes in cartilage metabolism and does not always correlate with the most significant clinical expression in OA, pain. The purpose of this study was to investigate the associations between joint metabolism and inflammation with pain and physical function in adults with painful knee OA. This is in support of qualifying burden of disease biomarkers with clinical variables to support the study, management, and understanding of disease pathology in OA.
Methods: The study used baseline data from 54 adults with knee OA participating in a nutrition intervention study. The 100 mm visual analogue scale version of the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) was used to assess pain, stiffness, and physical disability. A minimum WOMAC pain score of 125 was used as a cutoff for participant inclusion. Physical function was assessed with a 6-minute walk test (:6MWT) and stair climb task (SCT). Serum concentrations of biomarkers included measures of cartilage degradation (cartilage oligomeric matrix protein [COMP]), cartilage synthesis (type-IIA collagen N-propeptide [PIIANP]), synovial metabolism (hyaluronic acid [HA]), cartilage degrading enzyme levels (matrix metalloproteinase 3 [MMP-3]), and inflammation (C-reactive protein [CRP]). COMP, PIIANP, HA, and MMP-3 were measured by enzyme-linked immunosorbent assay and CRP was measured by an immunoturbidimetric assay. Correlations between biomarkers and clinical variables were assessed using Spearman correlation coefficients.
Results: Participants (38 females and 16 males) had a mean age of 60 $\pm$ 11.9 years, BMI of 32.6 $\pm$ 7.5 kg/m2, disease duration of 86.6 $\pm$ 112.2 months, and WOMAC pain score of 193.4 $\pm$ 94.04 mm. Higher serum MMP-3 levels were significantly associated with higher WOMAC pain scores ($R = 0.27$, $p = 0.05$), controlling for age and body mass index (BMI). Higher levels of serum HA were significantly associated with decreased walking distance in the :6MWT ($R = -0.35$, $p = 0.01$) and longer time taken in the SCT ($R = 0.31$, $p = 0.02$), when controlling for age and BMI. Higher CRP levels were significantly associated with higher WOMAC physical disability score ($R = 0.34$, $p = 0.01$) and worse performance in the :6MWT ($R = -0.38$, $p = 0.005$) and SCT ($R = 0.44$, $p = 0.001$) when age was controlled for, but these associations were not significant when BMI was controlled for. COMP and PIIANP were not significantly associated with any clinical variables analyzed.
Conclusion: Serum MMP-3 levels were associated with pain and serum HA levels were associated with physical functioning in adults who suffer from mild to moderate pain and impaired physical functioning from knee OA. Previous studies have shown that HA and MMP-3 are related to structural knee parameters in OA. Therefore, results from this study demonstrate that serum HA and MMP-3 also have potential as qualified burden of disease biomarkers that are clinically meaningful. (Research supported by the Ontario Ministry of Agriculture, Food and Rural Affairs, project #200121).

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FAST FIELD-CYCLING NMR OF CARTILAGE: A WAY TOWARD MOLECULAR IMAGING

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Purpose: A previous pilot study (presented at OARSI 2012) showed that Fast Field-Cycling NMR (FFC NMR) can be used to characterise the dispersion curves of cartilage in the region 0.4 to 3 MHz proton Larmor frequency. One feature of these dispersion curves, quadrupolar peaks, arise from relatively well known interactions between water protons and the ^{14}N nuclei of certain immobilised proteins. We have also previously shown that osteoarthritic cartilage gives rise to smaller quadrupolar peaks than cartilage from healthy volunteers. However, the exact protein responsible for the quadrupolar peaks observed in cartilage samples is uncertain. This present work aims to determine the protein responsible for the quadrupolar peaks observed in cartilage and how these signals correlate with disease progression.
Methods: Cartilage samples from femoral heads and knee joints were obtained after consenting patients undergoing joint replacement surgery or above-knee amputation at NHS Grampian Hospitals. All work with human tissue was approved by the North of Scotland research ethic committee. First, a pilot study was conducted on a commercial FFC NMR scanner (Stelar s.r.l, Mede, Italy). We used a pulse sequence with a short acquisition time ($<$ 1 ms), and included 7 patients undergoing arthroplasty for osteoarthritis (OA) and 5 patients undergoing hemiarthroplasty for hip fracture. The hip fracture group had no clinical or radiological evidence of OA prior to the fracture being sustained and there was no macroscopic evidence of cartilage degeneration seen intra-operatively on femoral head inspection. In a second, larger study, we examined the cartilage from 50 patients with evidence of OA changes and 50 without using both long ($\sim$20 ms) and short acquisition times.
We also measured the quadrupolar signals from a variety of samples using both long and short acquisition times including: normal and osteoarthritic human cartilage; collagen preparations using distilled water and a commercially available porcine collagen sponge (Collatamp; Tribute pharamaceutical); glycosaminoglycan extracts from human cartilage (both liquid and lyophilised); and human cartilage which had been extracted of its glycosaminoglycan (GAG) content with 4M guanidinium chloride.
Results: Variations of the quadrupolar signals were visible between short ($<$ 1ms) and long (CPMG echo trains, 20 ms) acquisitions. Long acquisitions did not show any contrast between normal and diseased cartilage whereas significant differences in quadrupolar peak amplitude were observed using short acquisition time sequences ($3.6\ s^{-1}$ vs $2.2\ s^{-1}$, $p < 0.01$). We observed no quadrupolar peaks in glycosaminoglycan extracts from cartilage, whether liquid or lyophilised.
Preparations containing 50%w/w collagen showed quadrupolar peaks indicating that collagen may be the source of this signal in cartilage. However, the quadrupolar peaks observed using short acquisition times were only seen in cartilage which had not been extracted of its GAG content. This suggests that the quadrupolar peaks observed in cartilage are likely to be linked to the macromolecular collagen fibril network and are reduced with decreasing matrix integrity. Interestingly, the short-lived component of the FFC-NMR signal was not evident in GAG-extracted cartilage cores from healthy or diseased cartilage. However, the long-lived signal was unaffected and was not found to be correlated with OA changes.
Conclusions: GAG extracts of cartilage samples and analysis of collagen preparations have shown that GAGs are not likely to be the proteins responsible for quadrupolar peaks. The amide linkages on the backbone of collagen are therefore indicated as the source of quadrupolar peaks but the organisation of the macromolecular collagen network within the cartilage matrix also seems to play an important role in the interaction between collagen proteins and water protons.
When this matrix is disturbed, such as after GAG-extraction, the amplitude of quadrupolar peaks are significantly diminished and there is a loss of the short-lived FFC NMR signal. Therefore it is likely that the quadrupolar signals of cartilage report on the macromolecular